

STIGMA RECEPTIVITY TEST IN DIVERSE SPECIES OF TOMATO

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ABSTRACT

The present experiment on tomato comprised of 17 genotypes belonging to ten different species. The research was carried out at "Research Centre for Distant Hybridization in Field and Fruit Crops, Dept. of Agricultural Biotechnology, Anand Agricultural University, Anand during 2016-2017". Stigma receptivity being one of the important traits influencing the rate of pollination and successful fertilization was under taken in the present study. The observations were recorded from tomato species on stigma receptivity by using Hydrogen peroxide. The stigma was found receptive before 12 hrs. of anthesis and may continue upto withering stage of flower, which was the common pattern observed in all the species. Stigma became fully receptive after opening of flower bud. But, in case of accession LA 1777 (*S. habrochaites*), the stigma did not become receptive at bud stage, while stigma of LA 2157 (*S. arcanum*) and LA 2819 (*S. lycopersicum*) showed receptivity from bud stage.

KEYWORDS: Anthesis, Stigma, Hydrogen Peroxide *S. Habrochaites*, *S. Arcanum* & *S. Lycopersicum*

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INTRODUCTION

The tomato belongs to the family *Solanaceae* (also known as the nightshade family), genus *Solanum*, subfamily *Solanoideae* and tribe *Solaneae*. Tomato (*Solanum lycopersicum*) is a major fruit crop with great economic importance worldwide that is used for both fresh consumption and processing. It provides many benefits to human nutrition and health. In addition, tomato has become a model plant in a wide range of research disciplines, including genetics (Chetelat *et al.*, 2000; Tanksley, 2004), evolution (Peralta *et al.*, 2005; Moyle, 2008), and reproductive biology.

Presently, there are 16 wild species of tomato including *S. habrochaites*, *S. pennellii*, *S. pimpinellifolium*, *S. cheesmaniae*, *S. galapagense*, *S. peruvianum*, *S. corneliomulleri*, *S. chilense*, *S. chmielewskii*, *S. arcanum*, *S. neorickii*, *S. huaylasense*, *S. lycopersicoides*, *S. ochranthum*, *S. jugandifolium* and *S. sitiens* (Knapp *et al.*, 2003; Bedinger *et al.*, 2011). These all species in the tomato clade are diploid with the same chromosome number ($2n = 2x = 24$) and are considered to have evolved primarily by genic changes rather than large-scale chromosomal rearrangements (Anderson *et al.*, 2010).

Stigma receptivity is a crucial stage in the maturation of a flower which may greatly influence the rate of self-pollination, pollination success at different stages in the flower life cycle, the relative importance of various pollinators, the interference between male and female functions, the rate of competition via improper pollen transfer, and the chances of gametophytic selection (Galen *et al.*, 1987). The information about duration of stigma receptivity may be very well utilized for making crossing or hybridizing program successful. Receptive stigmas

are characterized by high enzymatic activity. The presence of several enzymes is found to coincide with this developmental stage (Knox, 1984; Shivana and Rangaswamy, 1992) and consequently most of the methods to determine stigma receptivity *in vitro* are based on the identification of enzymatic activity (Knox *et al.*, 1986; Dafni 1992; Kearns and Inouye, 1993). This paper presents a known method for stigma receptivity using hydrogen peroxide (6%) in tomato.

MATERIALS AND METHODS

Stigma receptivity was studied by hydrogen peroxide test under microscope in a laboratory. The flowers were taken at three stages *viz.*, before a thesis (bud stage), full bloom and withering stage. The stigmas from flowers were kept on cavity slide then hydrogen peroxide (6%) was dropped on it. It was observed under zeiss microscope at 10 X and 40 X microscopic fields. The bubbling from stigma was considered as mark of receptivity of stigma. The observations were recorded in 1 hour interval from 8 a.m. to 8 p.m.

RESULTS AND DISCUSSIONS

The pistils were long and thin in wild species and the stigmas were exposed on the outside of the anther cone. The light green stigmas seemed somewhat swollen and were difficult to distinguish from the connecting styles. In contrast, the stubby pistils of cultivated genotypes were entirely embedded within the anther cone and the conspicuous capitate stigma was distinct from the style.

The observation regarding this trait was taken from accessions of all species taken under this experiment, which are given in **Table 1.1** and **Figure, 2-3**. A solution of hydrogen peroxide (6%) was placed on the stigma and the appearance of bubbles was observed under microscope and scored. The test was carried out in diverse tomato species at discrete classes of age *viz.* (A) bud stage, (B) freshly opened stage and (C) withering stage to determine period and duration of stigma receptivity. The reactivity was scored based on the extent of bubbling. From the present study, it was observed that in all the species, stigma became receptive one day before opening of flower and remained receptive up to withering stage. However, in some species *viz.*, LA 2157 (*S. arcanum*), LA 3859 (*S. pimpinellifolium*), LA 0490 (*S. lycopersicum*), LA 2817 (*S. lycopersicum*), LA 2819 (*S. lycopersicum*), LA 4440 (*S. lycopersicum*) and GT-2 (*S. lycopersicum*) the bubbling was also found higher in withering stage.

In contrast, at bud stage, very low reactivity was scored by LA 1777 (*S. habrochaites*) while LA 2157 (*S. arcanum*) and LA 2819 (*S. lycopersicum*) scored moderate and high reactivity, respectively. The above results revealed that in LA 1777 (*S. habrochaites*) the stigma did not become receptive at bud stage while stigma of LA 2157 (*S. arcanum*) and LA 2819 (*S. lycopersicum*) showed receptivity from bud stage. The receptivity of stigma increased gradually in bud stage as after 5.00 pm *i.e.* before 12 hrs. Of anthesis, which was the common pattern observed in all the species under study. Stigma became fully receptive after opening of flower bud.

Stigma of older flowers (C) which tested positive for peroxidase activity showed a varying level of reaction. LA 3652 (*S. lycopersicum* var. *cerasiforme*), WIR 13708 (*S. lycopersicum* var. *cerasiforme*), LA 2556 (*S. hirsutum*), EC-52005 (*S. hirsutum*) and EC-520047 (*S. chilense*) showed lower bubbling while WIR 5032 (*S. chilense*) and EC-520047 (*S. chmielewskii*) showed lesser extent of bubbling. In some accessions *viz.*, LA 2157 (*S. arcanum*), LA 3859 (*S. chilense*), LA 0490 (*S. lycopersicum*), LA 2817 (*S. lycopersicum*), LA 2819 (*S. lycopersicum*), LA 4440 (*S. lycopersicum*) and GT-2 (*S. lycopersicum*), the reactivity was found higher in C stage as observed in the B stage of flower in other genotypes.

The higher activity of hydrogen peroxide, suggested that the H_2O_2 is predominant in stigma. The H_2O_2 or reactive oxygen species (ROS) found in most of the plants as well as animals which play a diverse role in cell signaling, where they regulate diverse aspects of plant metabolism and cell growth. The H_2O_2 found to be involved in hypersensitive response during pathogen attack and programmed cell death (Bolwell, 1996; Blee *et al.*, 2001; Bolwell *et al.*, 2002). At withering stage, the cells might have gone through programmed cell death leading to accumulation of H_2O_2 / ROS which showed more bubbling.

Sidhu *et al.* (1980), Kalloo (1991) and Kaul (1991) studied stigma receptivity in tomato and reported that in tomato, stigma becomes receptive 16 to 18 hours before anthesis and retains the receptivity upto six days after anthesis.

Further, the reliability of peroxidase test was supported by Dupui & Dumas, 1990 and Mc Inni *et al.*, 2006, who reported that, the receptive stigmas 'ripe' for pollination and are characterized by high levels of peroxidase activity and these tests are widely used to determine pistil receptivity measuring stigma peroxidase activity (Dafni & Maues, 1998).



Figure 1: Stages of Flowers Collected for Stigma Receptivity

Table 1.1 Stigma Receptivity by Hydrogen Peroxide (H₂O₂) in Various Cultivated and Wild Species / Accessions of Tomato

Sr. No.	Genotype	Species/ Genotypes	Stage	8am-9am	9am-10am	10am-11am	11am-12am	12noon-1pm	1pm-2pm	2pm-3pm	3pm-4pm	4pm-5pm	5pm-6pm	6pm-7pm	7pm-8pm
1	LA 3652	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	A	-	-	-	-	+	+	+	+	++	++	++	+++
			B	++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	-	+	+	++	++	+	+	+	+	+	+	+
2	WIR 13708	<i>S. lycopersicum</i> var. <i>cerasiforme</i>	A	+	+	+	-	+	-	+	+	-	-	++	++
			B	++	++	++	+++	++++	++++	++++	+++	+++	+++	++++	++++
			C	+	+	-	-	-	+	+	+	+	+	++	-
3	LA 2556	<i>S. hirsutum</i>	A	+	+	-	-	-	+	++	+++	+	+++	+++	+++
			B	++	++	++	+++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+	+	+	+	++	+	+	+	+	+	+	+
4	EC-520058	<i>S. hirsutum</i>	A	+	+	+	+	+	++	++	++	++	+++	+++	+++
			B	+++	+++	+++	+++	++++	++++	++++	++++	++++	++++	+++	+++
			C	+	+	+	++	+	+	+	+	+	+	+	-
5	EC-52004	<i>S. cheesmaniae</i>	A	+	+	+	+	+	+	+	+	+	++	+++	+++
			B	++	++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++
			C	++	+	+	+	+	+	+	+	+	+	+	+
6	WIR 5032	<i>S. chilense</i>	A	+	-	+	+	++	-	+++	+++	++	++	+++	+++
			B	++	++	++	++	+++	+++	+++	+++	++++	++++	+++	+++
			C	++	++	++	++	++	+	+	+	+++	++	+++	+++
7	EC-520047	<i>S. chmielewskii</i>	A	+	+	+	+	+	+	+	+	+	+	+	+
			B	++	++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+++	+	++	++	++	+	+++	+++	+++	++	+++	+++
8	IIHR 1970	<i>S. peruvianum</i>	A	-	-	-	-	-	-	-	-	-	+	++	+++
			B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+++	+++	++	++	++	++	++	++	++	++	++	++
9	LA 1777	<i>S. habrochaites</i>	A	+	+	-	-	-	+	+	+	++	++	+++	+++
			B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+++	+++	+++	++	+	+++	-	-	++	+++	-	-
10	LA 2779	<i>S. chilense</i>	A	+	+	-	+	+	++	++	++	+	++	++	++
			B	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
			C	+++	+++	+++	+	+++	-	+++	+++	++	++	-	-
11	LA 2157	<i>S. arcanum</i>	A	+	+	-	-	++	++	++	++	++	+++	+++	+++
			B	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	++++	++	+	++	+++	+++	++	++	+++	+++	+++	+++
12	LA 3859	<i>S. pimpinellifolium</i>	A	-	+	+	++	+	++	+++	+++	++	+++	+++	+++
			B	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	++	-	+	+++	+++	+++	+++	+++	++	++	++	++
13	LA 0490	<i>S. lycopersicum</i>	A	+	++	+	+	+	+	++	++	++	+++	+++	+++
			B	++	+++	+++	+++	++++	++++	++++	++++	++++	++++	+++	+++
			C	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
14	LA 2817	<i>S. lycopersicum</i>	A	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++
			B	++	+++	+++	++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	++++	++++
15	LA 2819	<i>S. lycopersicum</i>	A	+	++	+++	+++	+++	++	++++	++++	++	++++	++++	++++
			B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	+++	+++	++	+++	+++	++++	+++	+++	+++	+++	+++	++++
16	LA 4440	<i>S. lycopersicum</i>	A	-	-	-	+	++	-	+	+	++	++	++	+++
			B	++++	+++	++++	+++	+++	++++	+++	+++	+++	+++	+++	+++
			C	+++	+++	+++	+++	++++	+++	+++	+++	++	+++	+++	+++
17	GT-2	<i>S. lycopersicum</i>	A	-	+	++	+	++	+	++	++	+	++	+++	+++
			B	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++
			C	++	++	++	+++	++++	+++	+++	+++	+++	+++	+++	++

A	Bud stage	-	No reactivity
B	Full bloom stage	+	Very less reactivity
C	Flower withering stage	++	Less reactivity
		+++	Moderate reactivity
		++++	High reactivity
		+++++	Very high reactivity

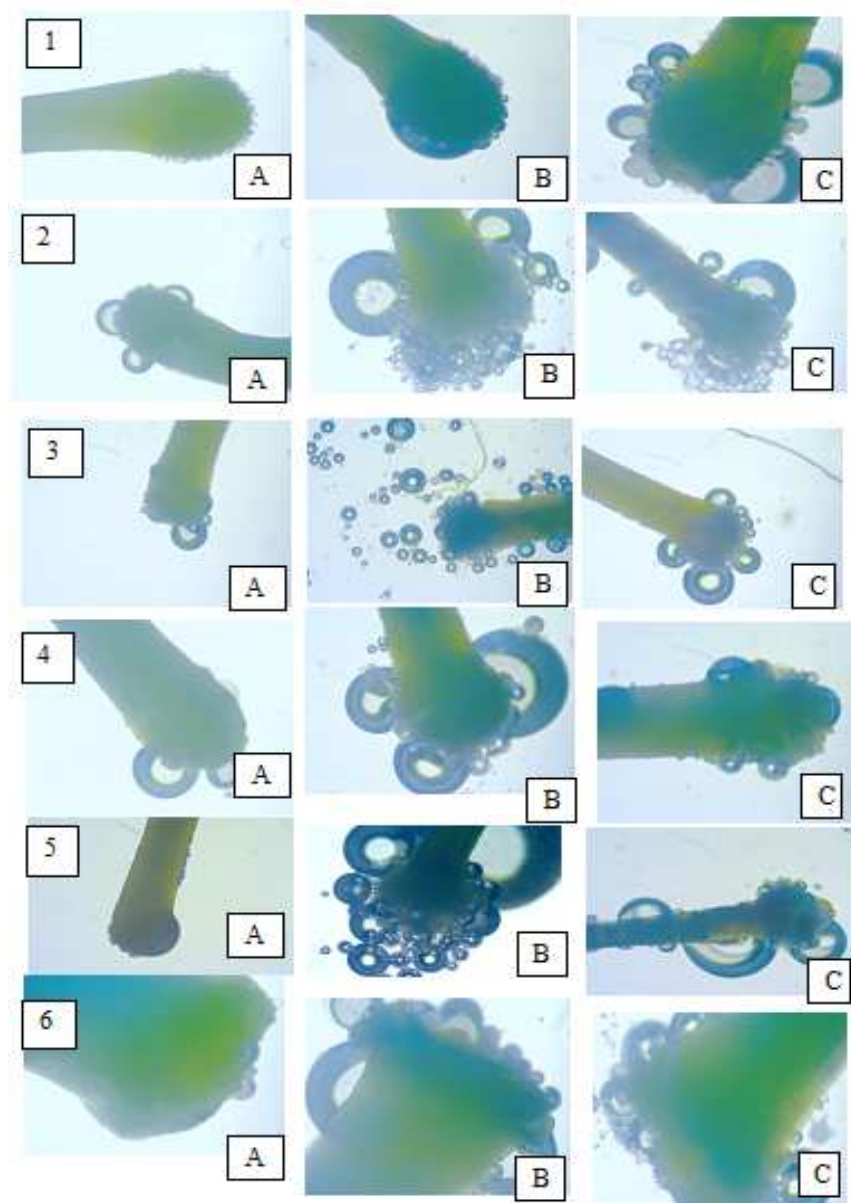


Figure 2: Stigma Receptivity using Peroxidase Test at Various Stages of Flower
(1-6)A= Bud Stage, B= Full Bloom Stage, C = Flower Withering Stage

1. LA 1777 (*S. habrochaites*), 2. LA 1970 (*S. peruvianum*), 3. WIR 5032 (*S. chilense*), 4. EC-520047 (*S. chilense*), 5. LA 2157 (*S. arcanum*), 6. LA 0490 (*S. lycopersicum*), 7. LA 4440 (*S. lycopersicum*), 8. GT-2 (*S. lycopersicum*), 9. LA 3652 (*S. lycopersicum* var. *cerasiforme*), 10. LA 2556 (*S. hirsutum*), 11. EC-52004 (*S. cheesmaniae*)

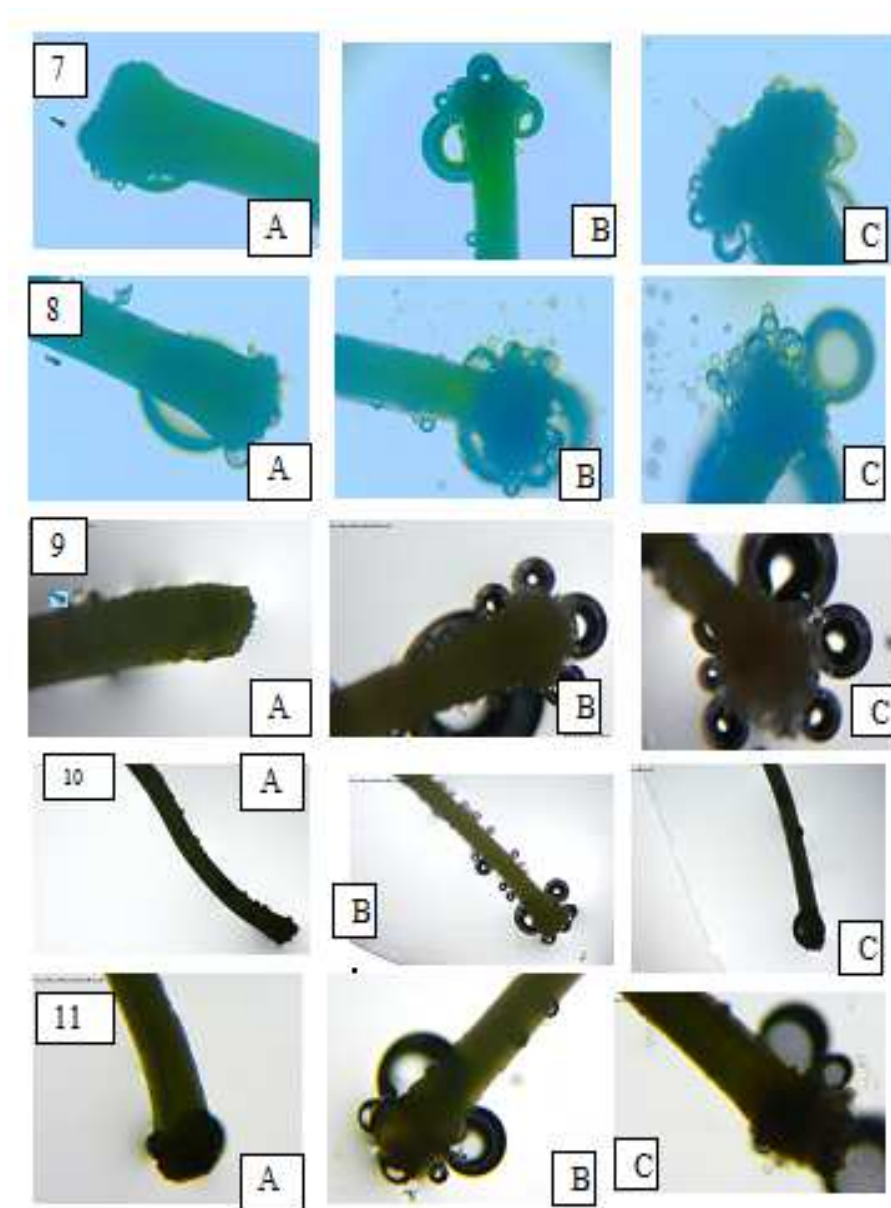


Figure 3: Stigma Receptivity using Peroxidase Test at Various Stages of Flower (7-11)
A= Bud Stage, B= Full Bloom Stage, C = Flower Withering Stage

1. LA 1777 (*S. habrochaites*), 2. LA 1970 (*S. peruvianum*), 3. WIR 5032 (*S. chilense*), 4. EC-520047 (*S. chilense*), 5. LA 2157 (*S. arcanum*), 6. LA 0490 (*S. lycopersicum*), 7. LA 4440 (*S. lycopersicum*), 8. GT-2 (*S. lycopersicum*), 9. LA 3652 (*S. lycopersicum* var. *cerasiforme*), 10. LA 2556 (*S. hirsutum*), 11. EC-52004 (*S. cheesmaniae*)

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